BRIEFER ARTICLES

PARTHENOGENESIS IN *PINUS PINASTER*

(WITH SEVEN FIGURES)

In the course of an investigation on the life-history and development of the embryo of the cluster pine, *Pinus pinaster* Soland., it has occasionally been noticed that in some ovules containing proembryos in all stages of development either no trace of pollen tubes could be seen in the nucellar cap, or the tubes only extended through a part of the nucellus and no nuclei could be found in them. This strongly suggested the occurrence of parthenogenesis, but might have been due to imperfect preparations. Therefore, in 1908, collections were made about every twelve hours during the time when the archegonia mature, in the hope of obtaining more satisfactory evidence.

Great care was used in fixing and imbedding this material, and the following fixing agent has been found more satisfactory than any other, including chromacetosmic mixtures.

Picric acid, saturated solution in 50 per cent. alcohol, 100^{cc}; corrosive sublimate 5^{gm}; glacial acetic acid 5^{cc}. This fixing agent is mentioned by CHAMBERLAIN,[1] and I have to thank Mr. A. J. BALLANTINE for suggesting its use. Cedar-wood oil has been found much superior to xylol to precede the infiltration with paraffin, as mentioned by Miss FERGUSON ('04). The stains used have been Delafield's hematoxylin, much diluted and allowed to act for several hours, and Flemming's safranin gentian-violet orange-G combination. The first named shows nuclear details more sharply than the triple stain and is only equalled in this respect by Haidenhain's iron alum hematoxylin, which is more troublesome to use and in no way superior. In other respects the methods used have been those generally employed in cytological work.

The evidence obtained shows clearly that parthenogenesis occasionally occurs, and the most conclusive preparations are shown in *figs.* 1 and 2.

The points which seem to prove satisfactorily that the oosphere develops without fertilization taking place are as follows:

1. Although the oosphere nucleus has divided or begun to divide, the pollen tube has not yet reached the archegonium and still contains both the sperm nuclei (*figs.* 1, 2).

[1] CHAMBERLAIN, C. J., Oogenesis in *Pinus Laricio*. BOT. GAZETTE **27**:268–280. *pls.* 4–6. 1899.

2. A careful study of the other sections of the series has failed to show any other pollen tube which might have reached the archegonium from another direction.

3. The spindle of the

EXPLANATION OF FIGURES

All sections 6–8 μ thick; cut with the Cambridge rocking microtome; drawn with camera. In all: *n*, nucellus; *p*, prothallus; *t*, pollen tube; *a*, archegonium; *v*, receptive vacuole; ♀, oosphere nucleus; ♂, functional sperm nucleus; ♂₂, secondary sperm nucleus.

FIGS. 1 and 2.—Stages in the first division of a parthenogenetic proembryo; diagrammatic; reconstructed from several sections in each case. ×77.

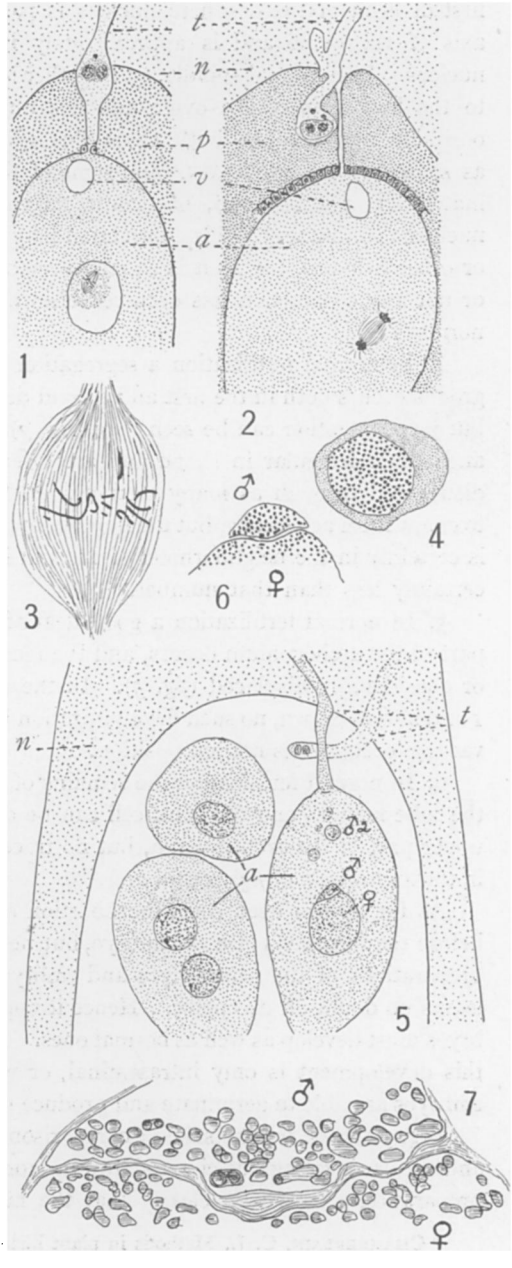
FIG. 3.—Spindle of *fig. 1*, showing the comparatively small amount of chromatin substance; drawn from two sections. (No attempt has been made to show all the chromosomes.) ×640.

FIG. 4.—One of the sperm nuclei of *fig. 1*, showing that these are quite normally organized. ×310.

FIG. 5.—Diagram showing normal fertilization in one of the archegonia. ×60.

FIG. 6.—Sperm nucleus and part of the oosphere nucleus of *fig. 5*. ×255.

FIG. 7.—Part of *fig. 6*. ×1240.



first division of the oosphere nucleus is parallel or oblique to the long axis of the ovule and is approximately in the center of the original nucleus. The normal spindle, on the other hand, is more or less transverse to the long axis of the ovule and lies quite at the top of the original oosphere nucleus. In both cases this spindle is entirely intranuclear, as shown by CHAMBERLAIN,[2] and some of the original achromatic nuclear material is not used up, but contracts considerably from the original nuclear membrane. (This contraction may be due to the action of fixing or other reagents, but as it is equally present in all preparations, whether or not any contraction has occurred elsewhere, I am inclined to think it normal.)

4. In normal fertilization a segregation of the chromosomes into two groups occurs both in the first and second divisions of the oospore nucleus, but no segregation can be seen here (*fig. 3*). The chromosomes are long and rather irregular in shape and are often cut into several pieces and distributed through as many sections. It has therefore been impossible to count them accurately, but the number in the normal sporophytic nucleus is certainly in the neighborhood of 24, and in the spindle of *fig. 3* it is as certainly less than that number.

5. In normal fertilization a good deal of disorganization of the apical part of the archegonium occurs, and the receptive vacuole is either broken or considerably displaced (*fig. 5*). In the archegonium from which *figs. 1* and *2* were drawn, no such disorganization has occurred, and the receptive vacuole occupies its normal position.

6. In normal fertilization the remains of the second sperm nucleus and the tube nucleus and the stalk cell can be distinguished for a time in the upper part of the archegonium, but no trace of these nuclei can be found in the archegonia of *figs. 1* and *2*.

As far as has been seen, the abortion of the ovule frequently occurs before the formation of a proembryo, but never after. A large number of preparations of the proembryos and embryo in all stages of development leaves no doubt on this point. Hence it appears that parthenogenetic embryos must develop as well as normal ones. It is impossible to say whether this development is only intraseminal, or whether seeds containing such embryos are able to germinate and produce normal plants.

Fig. 5 shows, for the sake of comparison, a case of normal fertilization, and the conjugating nuclei are shown in more detail in *figs. 6* and *7*. The preparation shows very clearly that the nuclear membranes are not in

[2] CHAMBERLAIN, C. J., Methods in plant histology. Second edition. The University of Chicago Press. 1905.

contact, but separated by a thin layer of cytoplasm. as mentioned by Miss FERGUSON.[3]

In *fig. 5* the second pollen tube is evidently on its way to the smallest of the three archegonia, and is taking its way laterally through the tissue of the prothallus instead of down the canal leading to the neck.

The third archegonium is apparently sunken in the tissues of the prothallus, but unfortunately the series is incomplete, and it may have only a very obliquely placed neck. For the same reason it is impossible to say whether this proembryo is really parthenogenetic, as it appears to be.—W. T. SAXTON, *South African College, Cape Town.*

CARNATION ALTERNARIOSE[4]

(WITH EIGHT FIGURES)

To a leaf-and-stem disease of the cultivated carnation, *Dianthus Caryophyllus* L., our attention was called by local florists as causing serious damage. The disease, upon examination, proved to be one hitherto undescribed and a laboratory study of it was undertaken.[5]

Symptoms.—The disease manifests itself as spots, mostly upon the leaves, sometimes upon the stems, especially at the nodes. These spots are strikingly characteristic, of ashen whiteness, with the center occupied by an often scanty, though sometimes profuse, black fungous growth. The diseased spot is dry, somewhat shrunken, thinner than healthy portions of the leaf, approximately circular, though often somewhat elongated in the direction of the longitudinal axis of the leaf (*fig. 1*). When occurring at the node, the disease usually involves the bases of both of the leaves, as well as the stem between them (*fig. 2*). As these nodal spots age, the disease penetrates through the stem, killing its tissue, which shrinks some-

[3] FERGUSON, M. C., Contributions to the knowledge of the life-history of Pinus, with special reference to sporogenesis, the development of the gametophytes and fertilization. Proc. Washington Acad. Sci. **6**: 1–202. *pls. 1–24.* 1904.

[4] This termination was suggested by the authors in Annales Mycologici **7**: 49. 1909, with the following explanation: "We believe that much will be gained both in clearness and brevity by designating diseases in plants by the uniform termination 'ose' (Lat. *osus*, signifying 'full of') added as a suffix to the genus of the causal fungus, with or without elision of the ultimate syllable of the generic name, in whole or in part, as may be determined by euphony."

[5] Through the kindness of Dr. W. A. ORTON of the U. S. Department of Agriculture, B. P. I., we learn that a Macrosporium disease of carnation was reported from Strassburg, Pa., in 1906, and one attributed to Alternaria from Connecticut by CLINTON in the same year.